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Synthesis and Characterization of N-N- {Bis(2′-Chloroethylamino)-Nitroso}-2-Benzylidene-1-Benzofuran Derivatives as Antileukemic Agents

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ABSTRACT

Aurones [2-benzylidine-1-benzofuran-3(2H)-ones] and its derivatives constitute a class of heterocyclic compounds have an extensive applications in drug development containing benzofuranone molecule. Aurones have been isolated from various natural sources and have been reported for their potent Anticancer, antidiabetic, anti-inflammatory activities. Alkylating neoplastic agents such as nitrogen mustards interfere with cellular proliferation. Based on the above consideration we have synthesized around eight benzofuran derivatives containing nitrogen mustard and screened for their In-Vitro Cytotoxic activity. Synthesis of N-N {Bis(2'-chloroethylamino)-Nitroso}-2-benzylidene-1-benzofuran was carried out by reacting O-hydroxy acetophenones with substituted benzaldehyde in presence of aqueous NaOH and was neutralized with 1N HCl to get chalcones, which was further refluxed with a mixture of $CuBr_2$ in DMSO and the reaction mixture was cooled, poured on ice-cold water to get aurones. Aurones was refluxed with hydroxylamine hydrochloride, sodium acetate trihydrate for 48 hours to get oxime, further treating with chloroacetyl chloride gave N-chloroacetyl-nitroso-2-benzylidene-1-benzofuran-3(2H)-one. This was further treated with Bis (2-chloroethyl) amine in pyridine to yield N-N {Bis(2'-chloroethylamino)-Nitroso}-2-benzylidene-1-benzofuran. The newly synthesized benzofuran derivatives were screened for their In-Vitro Cytotoxic activity against Breast cancer cell line. All the synthesized eight compounds showed mild to low cytotoxic activity.

Keywords: Benzofuran derivatives, alkylating agents, cancer, In-Vitro Cytotoxic activity.

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INTRODUCTION

Cancer accounts for about 23% and 7% deaths in USA and India. The total world's population by 2020 is expected to be 7.5 billion and around 15.0 million new cancer cases would be diagnosed; with about 12.0 million cancer deaths as predicted by approximations. The prevalence of cancer in India alone is estimated to be about 2.5 million, with about 7,00,000 to 8,00,000 new cases and 5,50, 000 deaths per annum.[1-3] Various studies reveal that Aurone, is a heterocyclic compound with flavonoid molecule with two isomers of the molecule, with both (*Z*)- and (E)- configurations. It contains a benzofuran element with a benzylidene molecule linked in position 2. It also constitutes, a chalcone like group with closed 5-membered ring. Approximately around 90-100 Aurones have been reported from natural sources, like in flowering plants, mosses, few ferns and marine brown algae. Aurones have been reported to have a pertinent biological activity such as anticancer, antileishmanial and antibacterial properties, Alzheimer's disease inhibitory activity against a variety of enzymes and proteins and have been developed as potential amyloid imaging agents.[4-7] Well proven nitrogen mustards are cytotoxic chemotherapeutic agent. Nitrogen mustards are one the promising and widely used alkylating anticancer agents which acts by alkylating DNA nonspecifically.[8] Even though various components are available as anticancer agents; Nitrogen mustards are proven as most potential alkalyting agent. Based on various studies and reports aurones are also known for their anticancer activity and each drug has its own side effects. In the view of all these, eight benzofuran derivatives were synthesized and screened for their *In-Vitro* Cytotoxic activity against Breast cancer cell line. All the synthesized eight compounds showed mild to low cytotoxic activity.

MATERIALS AND METHOD

Step-I: Synthesis of Chalcone.[9]

To a well stirred solution of substituted O-hydroxy acetophenone (50 mmol) and appropriately substituted benzaldehyde (50 mmol) in methanol (150 ml) was added a 50 % w/v aqueous NaOH solution (30 ml). The reaction mixture was stirred overnight at room temperature. It was neutralized with 1N HCl and filtered the product; incase of the absence of precipitate it was extracted with chloroform. The combined organic layer were dried (Na_2SO_4), filtered and evaporated. The product was purified by column chromatography.

IR spectrum (cm^{-1}): 3431(OH), 3063(Ar C-H), 1691(C=O), 1637(CH=CH) and 692(C-Cl). ^1H NMR ($CDCl_3$): δ 8.2(dd, 1H, =CH-Ar), 12.6(s, 1H, ArHC-Cl), 6.9-7.9(m, 4H, ArH), 8.6(dd, 2H, Ar CH-Cl).

Step-II: Synthesis of 2-benzylidene-1-benzofuran-3(2H)-ones.[10]

$CuBr_2$ (10-15 mg) was added to DMSO (10 ml) and 2'-hydroxychalcones (0.002 mol) were dissolved in this solution and the solution was refluxed for 1 to 1.30 hr. The reaction mixture was cooled, poured on ice-cold water and kept at room temperature for 10-15 min. The product was filtered and crystallized from ethanol.

IR spectrum (cm^{-1}): 3078(Ar C-H), 1693(C=O), 1637(CH=CH), 761(C-O-C) and 682(C-Cl).

^1H NMR ($CDCl_3$): δ 2.6(s, 3H, OCH_3), 6.9-7.9(m, 8H, ArH)

Step-III: Synthesis of 2-benzylidene-1-benzofuran-3(2H)-one oximes.[11]

Hydroxylamine hydrochloride (0.002 mol) and sodium acetate trihydrate (0.007 mol) were dissolved separately in hot water and to this was added the solution of aurone (0.001 mol) in alcohol (10-15 ml) and refluxed for 48 hr. The reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature to obtain the precipitate. It was filtered, washed with water, dried and purified by recrystallization from 90% ethanol.

IR spectrum (cm^{-1}): 3431(OH), 3082(Ar C-H), 1583(C=N), 754(C-O-C) and 657(C-Cl).

^1H NMR ($CDCl_3$): δ 1.2(s, 1H, N-OH), 1.6(s, 1H, CH-C-Cl), 3.7(dd, 2H, CH-CH-C-Cl), 5.4(s, 1H, CH=Ar), 7.0-8.0(m, 4H, ArH).

Step-IV: Synthesis of N-chloroacetyl-nitroso-2-benzylidene-1-benzofuran-3(2H)-one.[11]

Oxime (0.05 mol) was dissolved in 40 ml of 5% aqueous NaOH in cold and more water was added if necessary. If the solution was highly coloured, charcoal treatment was given. Then the solution was poured into 100 ml stopperd flask and to this was added 0.1 mol of chloroacetyl chloride and the reaction mixture was shaken vigorously until the odour of chloroacetyl chloride disappeared completely. The separated precipitate was filtered, washed with cold water and purified by recrystalisation from 90% ethanol.

IR spectrum (cm^{-1}): 3068(Ar C-H), 1608(C=N), 758(C-O-C), 669(C-Cl).

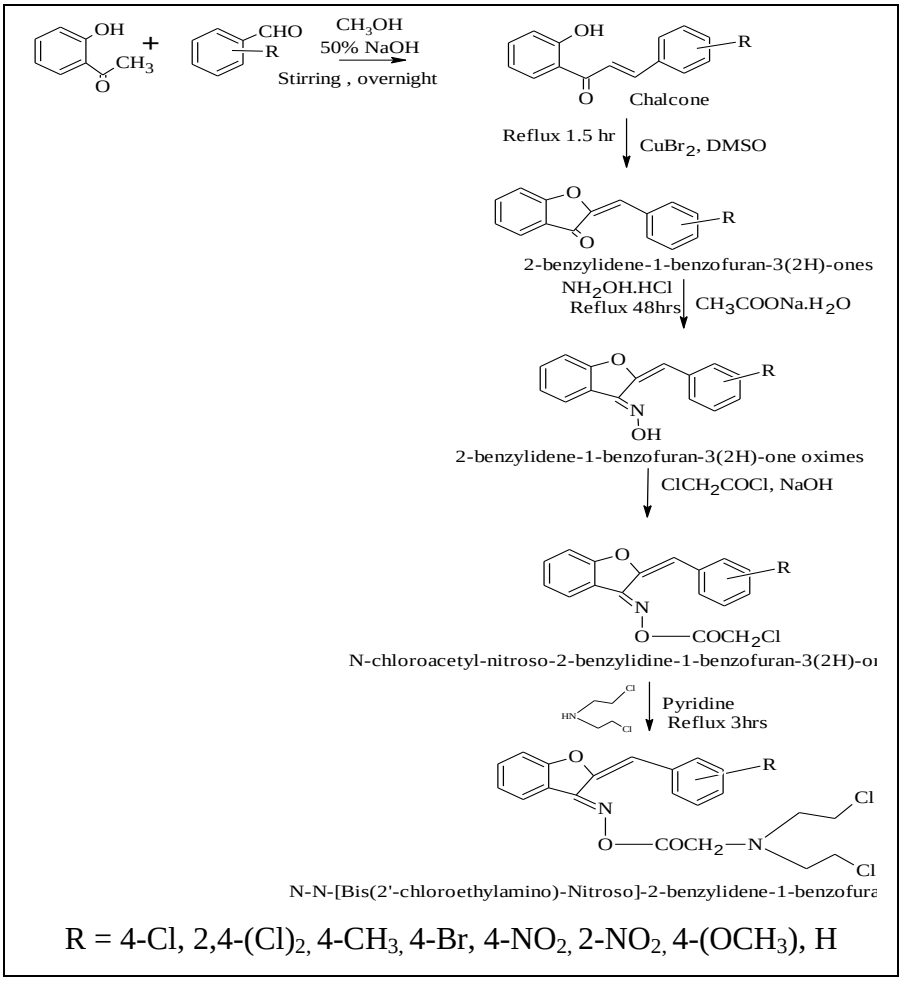
^1H NMR ($CDCl_3$): δ 2.7(dd, 2H, CH-Cl)2, 3.7(s, H, CHCl), 5.4(dd, 2H, -CH-Ar), 7.0-8.2(m, 4H, ArH).

Step-V: Synthesis of N-N-[Bis(2'-chloroethylamino)-Nitroso]-2-benzylidene-1-benzofuran.[12]

Chloroacetyl derivative (0.1 mole) was dissolved in Bis (2-chloroethyl) amine (0.012 mole) in pyridine and refluxed for 3 hrs. It was cooled and decomposed in ice water. The product was then filtered, dried and recrystallized using suitable solvent.

IR spectrum (cm^{-1}): 2924(Ar C-H), 1606(C=N), 1321(C-N), 707(C-Cl).

^1H NMR ($CDCl_3$): δ 1.2(s, 2H, CH_2Cl), 2.4-2.5(dd, 8H, $(CH_2\text{-}Cl)_2$), 3.7(dd, 2H, CH-CH-Cl), 5.4(dd, 1H, CH-Cl), 7.0-7.9(m, 4H, ArH).



Biological studies:

Cytotoxic study of synthesized compounds on Breast cancer cell line by MTT Assay. Cell lines and Culture medium Breast cancer cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO

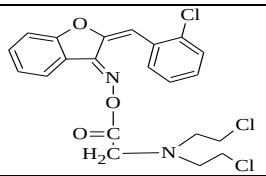
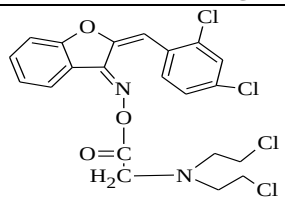
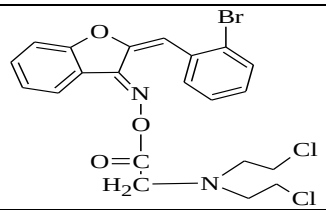
and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

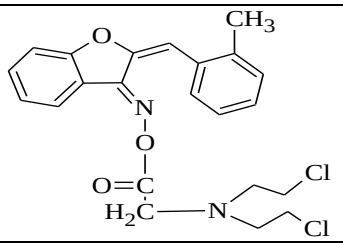
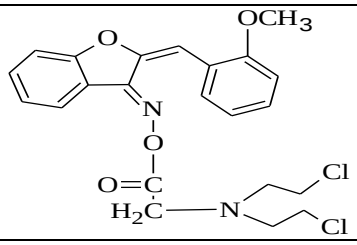
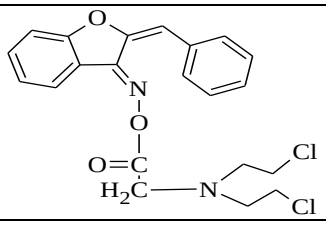
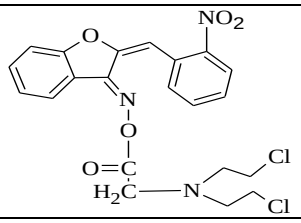
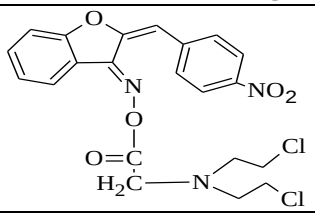
MTT or Tetrazolium salt Assay.

Initial treatment of cells with xenobiotics was carried out, the monolayers were genially washed with warm PBS using a multichannel pipette, and 100 µL of culture medium containing MTT solution (10:1) is added to each well. Some of wells without cells but incubated with MTT solution in order to have blank readings. Cells were kept for 1-3hr (depending on the cell density and activity) in the cell incubator. After incubation, the incubation medium was carefully removed. 100 µL of dimethyl sulphoxide was added and the plate was gently shaken to re suspend formed formazan, and waited until a homogenized colour was formed. Absorbance was taken at 490nm of wells containing cells and without cells (blank). The mean of absorbance was calculated after subtracting blank reading and control well was considered as 100% (maximum absorbance obtained) the results are expressed as percentage of inhibition. IC_{50} was calculated.[13]

RESULTS AND DISCUSSION

Eight N-N-[Bis (2'-chloroethylamino)-Nitroso]-2-benzylidene-1-benzofuran derivatives were synthesized by the above discussed methods and structures of newly synthesized derivatives was confirmed by FT-IR, 1HNMR and LC-MS spectral data.

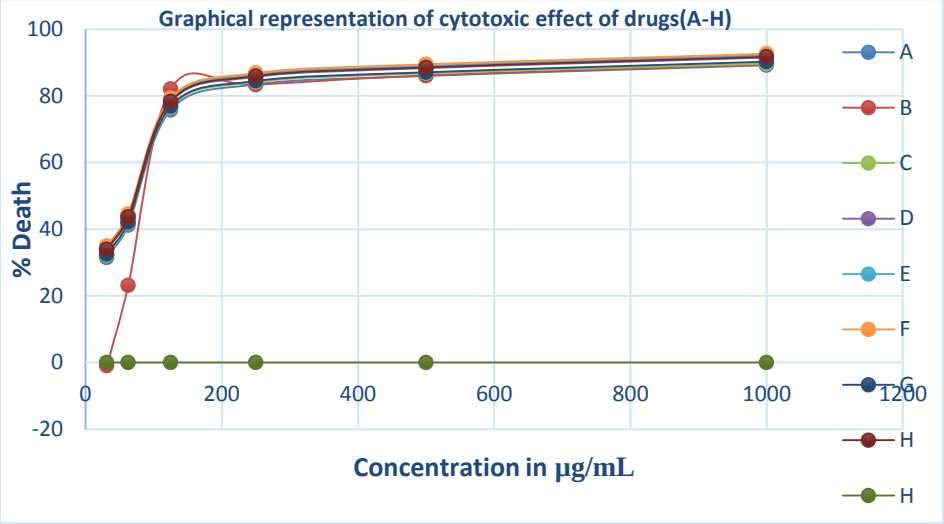
Compound Code	Structure	Molecular weight	%Yield	M.P ˚C
A		453.74	75	172-174
B		488.19	77	178-179
C		498.19	95	165-167

D		433.32	81	209-211
E		449.32	56	211-212
F		419.30	68	207-209
G		464.29	48	183-185
H		464.29	56	191-192

Biological evaluation:

In Vitro cytotoxicity was carried out by MTT assay on breast cancer cell line. All the eight compounds/drugs (A-H) have shown cytotoxic activity with IC50 values ranging between 62.64 to 89.04 µg/ml. Among the eight compounds (A-H), compound A (4-Cl), D (4-CH_3), E (4-OCH_3), F (H) and H (2-NO_2) exhibited mild cytotoxic activity. Whereas, compound B [2,4-$(Cl)_2$], C (4-Br) and G (4-NO_2) exhibited low cytotoxic activity.

Among all the eight drugs, drug F is the most potent of all with low IC50 value of 62.64 µg/ml. and drug B is the least potent of all with IC50 value of 89.04 µg/ml.



CONCLUSION

A series of nitrogen mustard linked benzofuaranone derivatives titled [A – H] have been synthesized using appropriate synthetic procedure. The yield of the synthesized compounds were found to be in the range of 48% - 95%. The structure of the synthesized compounds were confirmed by FT-IR, 1H NMR and Mass spectra. In vitro cytotoxicity studies were carried out on Breast cancer cell lines by MTT assay. All the eight compounds/drugs (A-H) have shown cytotoxic activity with IC50 values ranging between 62.64 to 89.04 µg/ml. Among the eight compounds (A-H), compound A (4-Cl), D (4-CH_3), E (4-OCH_3), F (H) and H (2-NO_2) exhibited mild cytotoxic activity. Whereas, compound B [2,4-$(Cl)_2$], C (4-Br) and G (4-NO_2) exhibited low cytotoxic activity. The newly synthesized N-N-{Bis(2'-chloroethylamino)-Nitroso}-2-benzylidene-1-benzofuran derivatives possess considerable cytotoxic activity and further lead optimization could be carried out for the better expected anticancer results.

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